11/5/53.

A. Mix ca 4 hours in Penassay. Plate out on EMB Lac.
Pk. (C.M.) Pick and streak Lac v colonies (ca 1% of total; on EMB Lac

Morphology (1 or 28)

	Lac	Mal	S	Mtl	Xyl	D(0) (ol⁻/lac)	Mal	S	Mtl	Xyl
1	+	— —	S	R	—	+	+ ↓	— ↓	+	
	+	—	S	R	—	+				
	+		R	R	+	+				
	+		R	R	+	+				
2	+		R	R	+				+	
	+		R	R						
	+		R	R						
	+		R	R						
3	+		R	R	+				+	
	+		R	R						
	+		B	R						
	+		R	R						
4	—	= no Xyl.	R	R	+ (no Xyl.)	+			+	no Mal
	+		R	R	+	+				
	+		S		—					
	+		S		—					
5	+		S	R	—	+		•+	•+ 28	28 no Mal
	+				—	+		+	+	
	+				—	+		•—	+ 1?	
	+				—	+		—	+	
6	+		R	R	+ +				+	no Mal
	+				+					
	+				+					
	+				+					
7	+	≠	S		—					
	+		S		—					
	+		R		+					
	+	omitted	R		+					

Morphology

W2057 = wg1 Hfr TLB₁ - Lac+ S^S Mal- Xyl- Mtl -
W2333 = wg28 F- Lac- S^R + + +

B. Repeat:

1076 A.
Summ.

W2333 × W2057.

IF— : Mtl-Mal-Mgl-S^S / +++ ^{R} completely linked.

13 Lac+ Im tested.

8:	Lac+ Mal+ / Lac − Mal+	R1/P1
4:	Lac+ Mal− / Lac+ Mal+ / Lac − Mal+.	R1/P1/P2
1:	Lac+ ~~Mal−~~ / Lac − Mal+	P1/P2

P2 type was morphologically distinguishable: lighter colour.

Note (as before) absence of ~~Lac~~ Mal − recombinants. Recovered P2 should be included for HFr (incl. ~~Hfr~~ Stellae). Also ~~with~~ morphology.

November 5, 1953.
W2057 + W1[illegible]

24h. Penassay cultures 1+1 + 5ml Penassay 4:40PM.—9:40.
Dilute [illegible] on EMBlac ± sm.

Test SR, Xyl, lac+/lac- for analysis

EMB[illegible]c sm. No SR+ at first. Ca 36 hours, weak lac+ appear (presumably Gal-).

A. EM[illegible]: from ca [illegible] plates, only 4 possible lac+/- at first.
Two gave lac+/-; two pure lac+ Test components as A1-2.
[illegible] Gal+/-. Later, more found and streaked out. Little likelihood of colony admixture, especially with lac+. lac- → lac+.

B. after ca 36 hours, weak lac+ appear, almost certainly diagnostic of lac+Gal-. Test s.c. from A, B.
↓
all pure Gal- in spot tests → 17: all S^R Mal+ Xyl+ Lac± Gal- # 2 MHl- + (15/16?) (11)

A1.

some v. tiny lac- colonies.

	Lac		Gal		S MHl Xyl Mal		
1	+ ✓	+	+	+	+, R	−
	−, +	+	−, +	+	+ R	−
		+		+		−
		+		+		−
2	−	+	−	+	+	−
	−	+	−	+	+	−
	−	+	−	+	+	−+?(R)
	−	+	−	+	+	
3	−, +	+	−, +	+	−, +R	−
		+		+		−
		+		+		−
		±		−		+ R
		±		−		+ R

A2.

	Lac	Gal	MHl / Xyl		Mal / S	
10	−	+ −	+ +	−+R	−+(R)
		± ✓	−	+	+ R
		± ✓	−	+	+ R
		+	+	−	− S
		+	+	−	− S
11	−+	± ✓ +	− +	+ +R	+ R
		± ✓	−	+	+ R
		± ✓	−	+	+ R
		+	+	−	− S

Addl. lac +, − from 2, 4, 6, 7, 8, 9, 11.
In 11, lac± verified. Others either ++ or − but Check on replica to EMBGal.

Aim of expt. is to determine the incidence of parent paratype and of Mal/Gal recombinants. These seemed more frequent than Lac/S.

A1 cont.

	Lac	Gal	Mal / Xyl / MH / SM	
4	−	+	−	+ R − S
	−		−	+ R
	+		−	+ R
	−		−	+ R
	−		−	+ R
5		+	+	−
		+	+	−
		+	−	+ R
		+	−	+ R
6	−	+	− +	+ R −
	−	+	− +	+ R −
	−	+	− +	+ R −
7	−	+	− +	+ R −
	−	+	− +	+ R −
	−	+	− +	+ R −
	−	+	− +	+ R −

	Gal	Lac	Mal / Xyl / MH / S	
8	−	+	−	+ R −
	+		−	+ R −
	+		+	−
	+		+	−
9	−	+	−	+ R −
	+		−	+ R − + MH, Mal
	+		+	−
	+		+	−

Numbers of 4-8 clones of A2 4 6 7 8 9 11, except in #11 are lac+, were Gal+ S^S lac−, Gal− S^R

Notes: 5: No lac⁻ clones in master but retrieved poss.: 3 clones seen on retrieval → Xyl to EMB Lac ± ones

Better procedure in lab notes for records. Might be to replicate as in 78A.

✓1: P1+P2−

5: P1+P2− seen +R1

4: Lac+ are Gal+ S^S (i were Lac+ Gal− S^R prob see

DATE: 11/14/53. REF: 1077 SUM.

A. Test for synkaryons. W2057 × W1321

Hfr TLB, Lac+ S^s Mal· Xyl· Mtl— ; Lac− Gal− S^R M−F−.

call. P2. ; P1 ; and R1 = P1 Lac+.

11 Lac v colonies: ✓further

1 P2 + P1 ✓ — Purity of A1−1, 2 + P1 + ? — Probably P+P2 ✓ + ~~...~~ P1/P2 from Lac v colonies?
2 P1, P2 ✓
3 P1, P2, R1
4 P1, P2 ✓
5 P2, R1 — − + P1 ✓ ~~...~~ P1?
6 P1, P2 ✓
7 P1, P2 ✓
8 P1, P2 ✓
9 P1, P2 ✓
10 P1, P2, R1
11. P1, P2, R1

~~Footnote~~ list:

bipar	7
bipar + R1	4
orthopar + R1	0

(not easily sought!)

no further record of presence of Lac ±: may have been present in some of these.

#5 Lac− and Gal− S^R = P1

B. Lac ±. No effort to identify Lac− components. 17 isolated were all Mal+ S^R Xyl− Gal− (orthotypic). 16/17 also Mtl−. Class B might have been favored by less restricted conditions for picking. [Gal and Mal-S generally concordant, but exceptions not yet looked for.]

Notes: defer more detailed analyses for single cell; Gal− Lac+ × Gal+ Lac−.

These results are now interpreted as synkaryons from which recombinants may or may not issue.

11/10/53.

Pinessay overnight (or 48 hours). 1:1:10 pinessay #2-5PM

A. W1895 x ~~W177~~. 1956. Plate on EMB lac ± sm. (E) 11/17 Check W1956 on EMB Mal OK.

B. W2057 x W1321 " "

C. W2057 x W2333

11/11 (D) W2058 x W1578. P12: no lac s noted. (Hfr??)

C:) Cram. 10^30 A11. 12 plates EMB lac + 2 fsm. No SR+ noted.
These plates have lac- > lac+, well separated colonies. Only well-isolated lac s/ picked for further study.

1- irregular margins. dark center, spatial edges. No definite sectoring.
No likelihood of contamination unless noted.
4,5 mucky fuzzy. 6 near lac- but not touching. 7 11 ~~[illegible]~~
9: def. sectoring 10 touching +. Disseminate.

P11. 12: small, fuzzy 13. (cont. c +) These colonies generally smaller than others. C.M. streak out from my pick

B) - hold E). lac+ >> Lac-. SR+ ca 5% of lac-. Lac+ too numerous for present purposes. SR+ to EMB Xyl. All Xyl-. By replica test, 9/19 were lac+ V₁ S, 10/19 lac+ V₁ R

P11 → B): Pick only lac s, whether weak or strong +/-, that are not likely contam. c lac+. Separate some possible adjunct +/-.
Pick only lac s that are not merely simple sector.
14 picked, all of type 15-20 are simple conjunctions.

A. EMB lac Lac s of two types: (1) and (2). Streak on EMB Mal
(VW1956 Mal!) 9/14 type 1 and 1/3 type 2 had Mal + (presumably the paratype parent. Restreak to analyse: spot s.c.i. lac+ and replica to EMB lac sm. (1 plate inadvertently [C.M.] streaked on EMB Sm: note several phenotypes!). (over)

Mal (in order)

A: +3

–1 – 4
–2 + 5
+ 6 + 13
+ 7
+ 8
+ 9
– 10
+ 11
– 12 ✓

– 14
+ 15
– 16

✓ c tacol n sequence used in streaks.

On EMB Lac, #2, 6 papillae

3, 6, 8 Lac+

13–16 on EMB Gal.

13 Gal++ > Gal– > Gal±
14. Gal± > Gal–
15. Gal± >> Gal–
16 Gal± > Gal±

	P2	R1	P1
	Lac+ S^s	Lac+ S^R	Lac– S^R
1	No	Rare ✓	✓
2	No ✓	✓	✓
3	✓✓	✓ occ.	✓ occ.
4	No	✓✓	✓✓
5	✓	✓✓	✓✓
6	✓ [Rare (secondary ?) →]	No!	✓✓
7	✓✓	✓	✓✓
8	✓✓	Rare sec. ? No	✓✓
9	No	✓	✓✓
10	No	✓	✓
11	✓	✓	✓✓
12	No	✓	✓✓
13	✓	✓✓	✓✓✓
14	No	✓✓	✓✓
15	✓✓	No	✓
16	No	✓	✓✓

Notes:

(Lac+ only papillae in orig.)

∴ assume these are in order by C.M.

Note v. many + to set ±.

Recheck for presence of Mal+! ~~Assign~~ O

If we accept 9 as P2 ✓ we have:

5 P1+P2+R1 : 3, 5, 7, (9), 11, 13,
3 P1+P2 (+R1??) : 6, 8, 15
7 P1+R1 : 1, 2, 4, 14, 16, 10, 12

A9 = Lac + Mal + S^R and Lac – Mal – S^R
+ Lac ± Mal + S^R

(P1+R1 not detectable), S^R mutant?

Restreak

1, 2 | Lac +
9 | Mal + → mostly Mal+ on restreak → ! which types on other medium!

11/16/53 Clean up A, B, C.

A9 Mal+ >> Mal− (1, or 2 - noted as streaks). Replica streak plate to EMB Lac ± sens. Mal− are lac− S^R (No S^S noted!) Mal+ are lac+ S^R lac− S^R

A1 Mal− only lac+ = S^R. P1+R1

A2 lac− only. (+ papillae) Mal− only. P1+R1

E: Mal Xyl−. Replica to EMB Lac T1.

B. 1 streak out possible Lac+ Sal− (11: ~~8~~: 1-4; 8:4)
both → lac±.

C. Plates heavily inoculated and overincubated hinders scoring lac+/−. 4,5 pure lac+ S^S 6: Lac+ S^R
inoculum mix scores: Repeat replica to lac, Mal, [redacted], Xyl, Mtl.

Concordance of Mal− Xyl− Mtl− S lac: Lac F
+ + + R − −
− − − S. + +

Mal →

	Lac−	Lac+	
1	3−	1+ 4−	R1 P1 P2
2	1−	3+ 4−	
3	4+	4−	
4		4−	
5		4−	
6	4+		
7	2+	3+ 3− (weak lac+)	
8	2+	3− 3 weak lac+ Mal+	
9	4+	4+	
10	4+	2+	
11		4−	
12	3−	3+	

	Lac− (orthotrip par.)	Lac+	
1	4+	3− 1+	P1 P2 R1
2	4+	3+ 1−	P1 P2 R1
3	4+	4−	P1 P2
4		4−	P2
5		4−	P2
6	[redacted] 4+		P1
7	[redacted]+	3− 3 Mal+ weak lac+	P1 P2 R1
8	~~4~~ 2+	3− 3 Mal+ weak lac+	P1 P2 R1
9	[redacted] 4+	4+	P1 R1
10	[redacted]+	4+	P1 R1
11	[redacted]	4−	P2
12	~~3+~~	3−	P1 P2

∴ 4 tritype + [redacted] < 3P2 1P1.
2 kiper
2 bitt ortho
Cf. R1 1,2 vs 7,8.

(over)

1078A9. (c) Lac+ Mal+ : Gal+ Xyl− MM− S^R

(b) Lac− Mal+ : Gal± " " "

(a) Lac− Mal− (1) : Gal± " " "

(a) = ω1177 = P1

(c) = R1 Mal+ } Mutation ?

(b) = P1 Mal+

Synkaryon tests.

DATE: 11/14/53. REF: 1078 S4M.

A. W1895 x W1956. Find Lac± 10/17 had a Mal+ component.

Test isolates for fraction also showing an SR+ component:

≡ Tentative conclusion: of 16 Lac+/−, ~~[illegible]~~ are biparental; ~~[illegible]~~ are biparental + orthotype recomb. and ~~[illegible]~~ are orthotype parent + recombinant.

Nutritional markers not tested.

B: W2057 x W1321. 14 Lac± isolated and types picked. [Adj. mixtures showed no Lac± instructed of this set!].

Lac± seen in following: (not recorded for 2 days!) 4 Lac+, 4 Lac− from each eye: #3 has Lac− only.

	+	±	−	
1	·		·	P1 P2
2		·	·	P1 P2
3		·	·	P1
4		·	·	P1 P2
5		·	·	P1 P2
6			·	? P1 P2
7		·	·	P1 P2
8	·	·	·	P1 P2
9				R1 P1 ~~[illegible]~~
10				"
11	·		·	P1 R1
12		·	·	P1 P2
13	·		·	"
14	·		·	"

Parentals only except poss: # ~~[illegible]~~ Lac+ Gal−
#8/4 " Restreak for check ✓

R1P ~~[illegible]~~.

Tent.: ~~[illegible]~~ bipar + ortho recomb.
~~[illegible]~~ bipar only.
~~[illegible]~~ par + ortho rec.
see 77A → 4:7

Total 77, 78: 6/19

In view of ambiguities, test replicas but do not pursue in place of forthcoming Gal-Lac x Gal+ Lac−

What tests? No record except for Lac, Gal, S.

C. W2057 x W2333 Lac+ and − except in 4, 5, 6, 11 (of spots +)
(4, 5, 11 had not been Lac ±). 12 n.g. 14 n.g.

P1.P2 colonies suggest that, judging from colony morphology units probably single cells are synkaryotic. Occurrence of recombinants strengthens the argument. (Look for V1/S recombinants?)

11/16/53.

ca. 1:1 in Synd broth 1[15] PM – 4[45] PM.

A. W1895 } x W2033 on EMB lac ± sm.
B. W2341 }

Lac+Gal− S^S × Lac−Gal+ S^R no easy way to detect genotype parent or Gal/S recombinants.

AA. lac+/− control AB: sectorial and pinpoint

A.C. fractions of parents. Replicate strokes to lac sm.

SR+:

AA. 46 colonies. 44 SR+ ~~~ 1 lac+ rare 1 lac+ inf. mostly S^S. Not certain whether secondary SR+ are completely controlled by AC. Note low proportion of P1+P2 in this entire experiment!

AB

10 probable P1+P2 (no or secondary SR+) / 36 total.

AC. 16 Spotty +/− only.

B. 9EMB lac (accurately plated .1 ml from 10^{-6} dil.) and 9EMB lac sm

On EMB lac, score all lac++ : almost all variegated with lac− or lac±
Scores may include some lac±/lac−. On EMB lac sm score lac+ and lac+/−. May include some S^R lac± but probably not.

BB.

	lac+...	lac−	Total	
Lac	1 23		551	
	2 24		534	
	3 ~~18~~		592	
	4 18	255	569	{255 are Gal+}
	5 22		563	
	6 34		545	
	7 19		576	
	8 19		516	
	9 22		560	
Mean:	22.1			

Σ 199

BA

	lac−	lac+	lac+/−	Total S^R
11		1	23	343
12		1	14	331
13		0	2* 22	308
14		0	33	302
15		2	22	295
16		1	33	320
17		1	18	335
18		0	23	328
19		0	24	345
Mean:			24.2	323.0
		6	212 / 218	

(only 2 under others →)

Averages not appreciably different. Unlikely that any genotypes are confused. I.E. SR+ are almost certainly lac+ Gal+ S^R. To identify Gal− would require separation of lac+ components and get total counts. All appear ++. Check pure + = 79 BB. (over)

EMBtal: low count of fuzzy tal+ or +/- colonies. 1-4/plate
= BC. scored, but not very distinctive

BB. On enriching, 2 were found to be pure lac+, others had rare lac-

Parent (means).

lac-	= 323.0	lac+ =	(545) 323
	- 14.2		-
	298.8		222.

BC: 15 tal +/-? or tal ±. Streak on EMB lac

	P1	P2	R1	
1	✓	✓	✓	
2.		✓	✓	sic = F3
3	–	–		
4	✓			
5	✓	✓	±	
6	✓	±	✓	
7	✓	✓	✓	
8	✓	✓	(±)	
9	✓	✓	±	
10	✓	✓	±	
11	✓	✓	±	
12	✓	–	✓	
13	✓	±	±	
14	✓	✓		
15	✓	✓	±	

1 R1+P2
3 P1+P2
10 P1+P2+R1

(1 P1)

EMB lac sen. Prop. yields of SR+ similar to 79B. 17/17 are sens lac+/- colonies
lac+ sectors usually small. Save for picture.

EMB lac. Numerous ⊕ colonies. All that can be learned here is the incidence of SR+ among these. Distinguish type 1 (A1-2) —, central + & radiations but surrounded by lac- and type 2 (A-3) — multisectored purple/lac+/- . On EMB lac sen, type 1 : type 2 = 7:5
12:9:1+
(Type 2 are less characteristic on EMB lac). Do not use this series (in preference to 79B) to test persistence of parapartial component.
Save some plates for photography. Note that lac+ recomb. are distinguishable here from W1895 lac+ also.

11/18. In replica from strokes, A3: virtually all had numerous SR+.
A1-2 not yet tested; also confirmt P1/P2 colony tests to be done.
Ca 9/36 had a lower incidence of SR+ than others. But this test is essentially too crude.

BAEMBlac. Mark clear +/- — and fuzzy +/±/- —
Pick only clearly isolated colonies. Among scorable, isolable colonies:
— are fac less conspicuous as
(and are lac±/- with
possible lac+ component)
isolable colonies.

BA 2	11	3
4	2	8
3	7	2
4	5	2
5	3	2
6	7	1
7	5	3
8	7	3
9	6	3
40		

an addnl. unnumbered plates:

11	2
1	1
6	3
7	7
4	2
1	0
2	0
7	1
3	0
5	2
1	2
3	0
5	4
9	4
5	3
2	1
4	2
9	2
7	2
92	38
D	E

Gal/lac "interaction" (EML Thesis) a prominent feature.

Parents: W2431 - pure Gal- Lac± W1895 Lac+ Gal+ W2033 lac- Gal+

E = BA yellow P 1

D = BA ~~red~~ ~~1-60~~ 1-136 (6.0.)

DATE: 4/18/53 REF: 1079

D. 101

	P1	P2	R1	4
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
110	✓	-	-	✓
	✓		-	-
	✓		-	-
	✓		-	-
	✓		✓	-
	✓		✓	-
	✓		✓	-
	✓		✓	-
	✓		✓	-
	✓		✓	-
120	✓		✓	-
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓	✓	✓	✓
-	✓			✓
	✓		✓	✓
30	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓		✓	✓
	✓	✓	✓	✓
	✓		✓	✓

∴ 12 are R1+P1

40 8 R1+P1+P2, though the low incidence of R1 suggests some of these are secondary.

1 R1 only.

1 P1

50

5	2 second 6	P_1+P_2 7	R_1+P_1	$R_1+P_1+P_2$	R_1+P_2
D (uncon.)			12 6	8	
E.	57	7	5	43 (20?)	2

D:E had been estimated at the sames actually found.

D+E		7	131	51	2

but there is a probable bias favoring the detection of R_1+P_1. (R_1+P_1) is evidently distinguishable from ~~R+P~~ ($R_1+P_1+P_2$) on original plates

P_1 = ~~Hfr~~ F- parent

P_2 = Hfr parent

R_1 = Recomb

Should tabulate incidence of R1 from conjugat $P_1 \cdot P_2$ colonies.

6/29 E: Pick 2 zones at junction of P_1 and P_2 in (and streak) (4). deistruct colonies. An EMBlac, no R1 in deistruct colonies. EMBlac con- SR+ types are papillae in their streaks. Thus deistruct from types refered to above as ✓ in R1 column.

Scores of streaks of 1079D on EMB Lac

DATE: 11/18/53 REF: 1079.

	P1	P2	R1	EMB Lac sm SR+ = R1	5	A1	A2	R1	9	10
D 1					51					
10					60					
20					70					
30					80					
40					90					
50					100					

P1 = F- Lac- wg 28

P2 = Hfr Gal- Lac+ wg 1

R1 = Lac+ Gal+

1079E
scores.

DATE: 11/18/53 REF: 1079D.

	P1	P2	R1	SR+	5	P1	P2	R1	9	10

least is marked at n1.

1

sic. → F1

sic. → F2

58.

∴ This series, selected for the identifiability of P2 in the colony, shows

6 + 1	P1 + P2
43 (23 certain + 20?)	P1 + P2 + R1
5	P1 + R1
2	P2 + R1 (= F)
1	P1

F: (1, 2, 3) (See also 10C). Save after verification of absence of P1. F1-2 also checked on EMB lac sm. No Lac− or Lac± here

(too effective in detecting recombinants)

✓ if Lac+ is considerable
All have at least papillate +
very few colonies in SR1 tests 1-33

1076-
1079.

	Notes.	P1+P2	P1+R1	P1+P2+R1	[redacted].
		1	8	4	Lac+/-
1076A	W2057 x W2333 sample colonies tested Mal MH Xyl-S concordant.				
Possibilities of syntheyon analysis concerned from this					
1077A	W2057 x W1321 EMBLac. Mal Xyl MH S concord.	7	0	4	Lac +/-
B	EMBlac sens. 17 all S^R Mal^+ Xyl^- Gal^- $Lac^\pm$ {16 MH- 1 MH+}.				
1078 A	W1895 x W1956 EMBLac. Test on EMB Mal for Lac+/- 9/16 [redacted] had Mal+	3	(+A9)	7 5	Lac+/-
	#9 had P1 & Lac+Mal+ S^R & Lac-Mal+ S^R				
Lac, Mal, S tested only.	(additnl. Recomb type : Mal X Son Mal+ recurrent?)				
1A: SR+: 9/9 V_1^S					
B.	W2057 x W1321	11	1	1	Lac+/-
Limited sample of colonies tested					
Lac, Gal, S.					
C.	W2057 x W2333	2	2	4	Lac+/-
Mal Xyl MH S concordant					
limited sample of colonies from each.					
1079 A.	W1895 x W2333. Test Lac+/- for SR+:				
	of. pinctatis.				
B	W2(4)31 x W2333. Also 2(R1+P2)	7	131	51 *	Lac++ associate
? * Maybe biased. Some R1 might be secondary, or many such colonies missed as not uni-cell origin.					
BC.	(P1+R1 of course not picked!). (+1P2R1)	3	0	10	Gal+/-

11/19/53

The general conclusion is that the Hfr parent is frequently associated with the F- in recombinant containing colonies. Etched question still whether these are unicellular in origin — considered like from the colony appearance ~~of~~ and from EKL findings.

It is difficult to calculate exactly what proportion of lac+/- colonies have Sk+ recombinants.

Single cell isolations of sypheomyces.

11/16/53.

ca. 11/10 ff. Preliminary trials.

A. Prep'n mercury droplets — overlooked Topley description. Had attempted to use needle directly; then used fine bore needle c syringe. Later found simple technique, and could pick droplet off glass plate by sliding needle + drop across the edge. Droplets ca ½ - ⅔ diameter of 43X field found adequate and could still permit study under phase contrast.

B. Insanity o.K. – Plastic coverglass shutting opaque. #1 coverglass: no killing even for 5 mins! Kodak film base allows partial transmission — req 3-5X normal dose, but poor optical quality. Visking absorbs water too readily.

C. Troubles: with several blocks: growth along and up sides. Peril of moving completed work by accidentally touching edge of coverglass

BB. Plastocel 10 ~~[illegible]~~ mils showed partial ~~transmission~~ (killing at 1 min dose) Maybe too scratched?

D. 11/22/53. After various casual efforts, try the method using 5 cm squares of agar blocks c plastocel squares about same size. 6 tests: 2 controls, 4 droplets. On controls, found 2, 1 colonies. On others, total of 26 but only 1 even close to a droplet. Complete failure!! UV dose may have been inadequate.

wg 28 Lac- Screen for test crossing markers.

1080

DATE: 11/18/13. REF:

W2341 × W1 × 1:1:5 12-N18.

A W2334

B W2335

C W2336

D W2337 - Strain already had lac+. (reversible?)

E W2338.

Recheck of these on EMB gal!

	lac V	SR+	EMB gal	EMB lac.
A	✓✓	—		—
B	no	no		flat, v. small colonies
C	✓✓	✓✓	no V	—
D				already some +
E	✓?	no		rather small

Use either A or C for future work. W2333 has the disadvantage of showing slow +.

Must be confused ↑. W2336 is Gal −, + mixed (of D)

Rec		Gal	lac
	W2333	+	− → ±
	4	+	− → ±
	5	+	−
	6	+, −	+, − Mal +, −
	7	−	− thin
	8	+	−

Use 3, 5 or 8. Try 8.

(over).

Motility.

W-2333-8, W1208A, W 2059 (wg 51) are non-motile under microscope, ~~43~~ 33 also by motility tube.

W1208 (hyaphil) is motile. W1258 (obl oval) is motile + (non motile) ?

8 A. W1258 o.v. small cols. (11/19 - 11/20)

1 B " " large.

"wg 28"

1081

11/22/53

W1258 = wg 28 = NCTC 123 as received from Cavalli. Is now microscopically motile; grows poorly on EMB; Lac+. Also should be S^S; λ^S; ... I. Re-isolate from lyophil 11/20/53.

W1258A = wg 28A. Recovered by EML from an old vial ~~#~~ 12/11/..?

~~Recorded~~ as prototrophic S^R. Mutants have morphology similar to that of wg 51 and are likewise also non-motile. Present stock wg 28A also non-motile. 2 types on EMB lac A1 = gummy. A2 - not

---

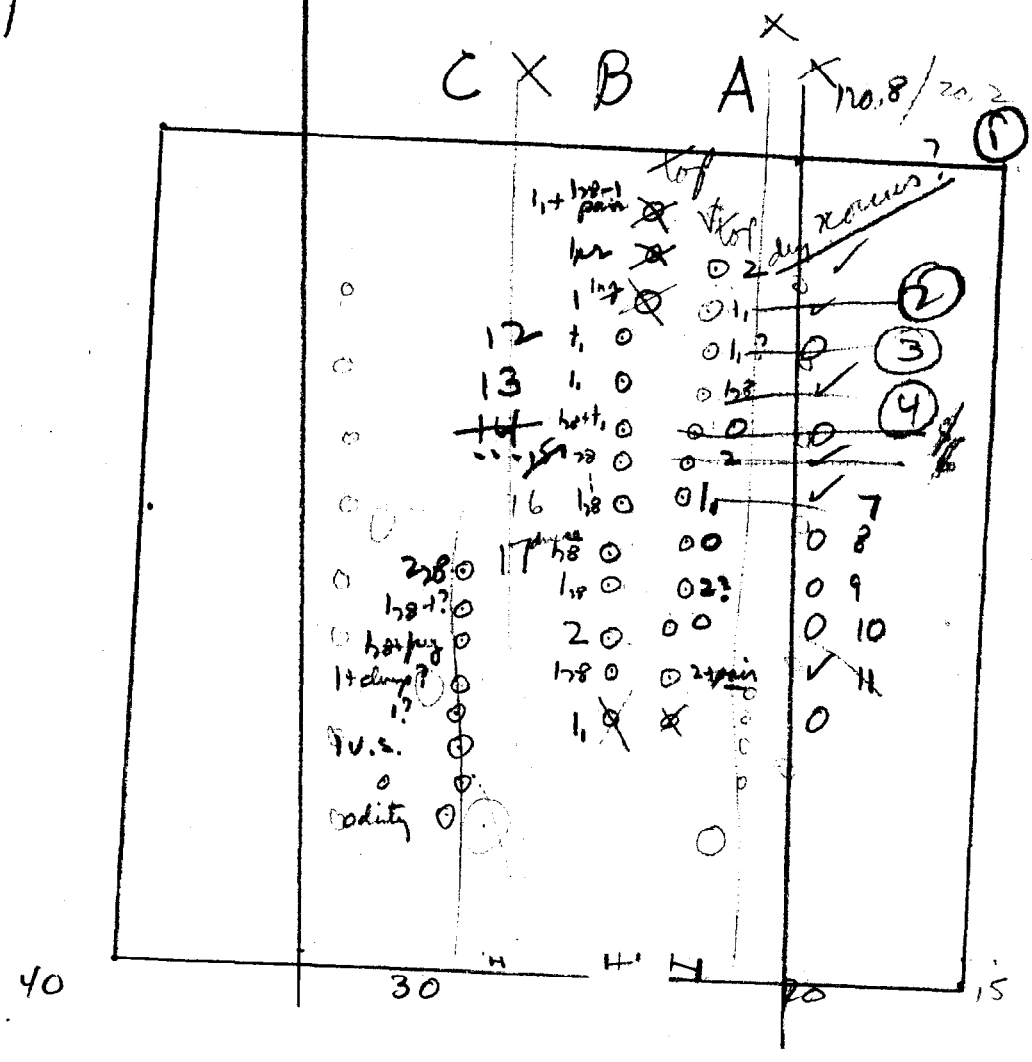
Old vial. Streaked out directly gave only lac- colonies (81A, B. both are distinctly motile. (Reisolated for presence of lac+ = 81D).

Added both, streaked out after growth mostly lac- S^R, as above. Occ. some lac+ S^S = 81C.

	Mot	lac	SM	T1	
1258	+	+ slow (s)			
A	+	−	R	R	R
B	+	−	R	R	R
C	wk +	+	S	R	R
wg 28A 1	−	+	(R)	R	R gummy.
2	−	+	(R)	R	S
2338...					S

1081

24 24-25 22-23



DATE: REF:

upper ... diagonal

some may have died.

	1	2	3	4	5	6	7	8	9	10
1-10 Exp-cells.	2 ✓	1, ✓	1, 0	1x8 ✓	=	=	1, ✓	0	2? 0	0 0
Growth (table)	✓ (mucus?)	?mucho —	✓	—			—	—	—	✓
type loc locus	P2 ✓	n.g. ✓	P1-R1 ✓							— R1-P2 ✓
11-20	2+pa.. ✓	+, ✓	1,	—	1+1	1x	1x8	1x8	2	1x8
Gr (table)	—	✓	✓	=	✓	—	—	✓	✓	—
type loc locus		~~P2~~	P1-P2 ✓		P1 only			P2 ✓	P1 ✓	

Segregation again is arguable

~~[illegible]~~

Some single cell possible

This expt. only explanation of mutated results n.g.

nodge

11/24/53. 2338?

A. W2344 (x W2341) 12:30 – 6 PM 1:1:10 Pen
(also use for oil chamber isolations (1:1000))
measured carefully.

1. EMBlac

2 EMBlac Sm (2X)

3 EMBlac Sm + T1 (2X) (20X)

4 EMBGal + T1. (2X)

B. ~~Single cells. Droplets 10^{-3} ml. in oil chamber. Study and consider 0, 1, >1 cell types. Allow to grow in chamber overnight. Pick~~

2: Brush lac+ S^R colonies (mostly also lac- S^R) / T1 on EMBlac
50 tested, 26 R lac+, 24 S lac+. All but one, if lac- present this was lac- V_1^S (unless masked by lac+ V_1^R).

1: lac+ V_1^S / lac- V_1^R = #18.

→ 1, 2, 3, 4, 5, 12, 13, 16, 17, (18), 19, 23, 24, 27, 28, 29, 31, 38, 40, 41, 43, 47, 48, 50

~~[illegible]~~:

∴ ca 50% of SR+ are V_1^R. (independence of Lac, V_1 here?) [Neither of the factors is known to be allelic with Lac_1, V_1 of line 1].

Au 1117 B

DATE: 11/25-26/53 REF:

A2. SR+ /plate = 22, 19, 23,

A3 20X 233+, 130−

2X [9+ 14−; 10+, 3−; 5+ 11−;] Includes 3 vertical columns.

Therefore there are an appreciable number of lac−V_1^R S^K., but no (Gal−lac+) $S^T V_1^R$ were noted in this experiment.

A4 (no T1) fuzzy colonies (total 18). Streak EMB lac for enzymes

	lac−	lac±	lac++		
1	✓	✓			
2	✓	✓	✓		
3	✓	✓	✓		
4	✓	✓	✓		
5	✓	✓	✓	12	P1+P2+K1
6	✓	✓	✓		
7	✓	✓	✓	2	P1+K1
8	✓	✓	✓		
9	✓	✓	✓	1	P2+K1
10		✓	✓		
11	✓	✓	✓		
12	✓	✓	✓		
13	✓	✓	✓		
14	✓	✓	✓		
15	✓	✓	✓		

+T1. Gal+ counts/plate 23, 21, 14, 12− (386 Gal−)

Phage probably inadequate for total immediate lysis. Cf #2/A3.

A2: lac+S^K = 21/plate., expect 11 to be V_1^R.

A3: ca ½ V_1^R formed, not in disagreement so no evidence of lag. But phage amount needs to be checked, also character of the lac−V_1^R S^K. Seems Gal+ V_1^R, comparable (perhaps low or heterogenotes) in S^T+.

Are notes 11/27/53

1/12/54

1082

T-do. a) Haploid crosses in independence of lac, T1.

b) Het diploids for ind. segr. of lac, T1 (unless linked to auxotrophies. Use SR+ if necessary)

Try S × M7

c) Transfer lfor to this stock by bal linkage. I.E.

→ find a bal⁻ V1^R recombinant in appropriate setups.

d) Study the bal⁺/bal⁻ ratio among lac+V1^R recombinants (direct scoring!)

e) look in frig.

There are 1/2 as many lac+S^R

There may will be many lac⁻V1^S recombinants (conducted along and with P1.

Some "P1+P2" might have such recombinants. (Either test at random or replicate cross plates.) For present work this means testing lac isolates on SM7 T1. Later study "P1+P2" in plates, and try to find P1+(R2). [R2 = lac⁻V1^S recombinant]

Also do (d) above for the record.

1/12/54 au1082

No data on V_1 distr. among lac^+ / lac^- .

$S^R lac^+ V_1^S$ usually accompanied by $S^R lac^- V_1^S$ $\left(\frac{20+}{24}\right)$ = P1

rather than $lac^- V_1^R$ = recipr. recombinant.

Should now test Gal^+ $\frac{lac^r}{ctg}$ colonies for comps. Cf. 1076-1079 Experiments probably nothing saved.

More likely to be with lac⁻ parent. I.E. $\frac{S^R Gal^+ lac^- V_1^S}{S^R Gal^+ lac^- V_1^R}$.

3 = recomb. classes 2 are lac+, now detected.

1 is $lac^- V_1^R$.

If associated with P1. not now detected as seg. colonies.
not readily detectable in segregates.

∴ all 1-cell isolates must also be scored on T1.

W2338 $V_1^R S^R$ (2344).

×

PH- Het $lac^+ Hal^-$ (Gal^-) m

allelism 2338 lac.

1/12/54

082. ? How many zygotes are mixed

a). Are there recombinants not detectable as $Lac^+ S^R$
b) Are there segregates other than Lac^+/Lac^-.

a. $234Y \times 234I = Lac^- S^R V_1^R \times Lac^+ Gal^- S^S V_1^R$
F^-

$Lac^+ S^R$ essentially all Gal+. 2 V_1^R 2 V_1^S.

S^R/V_1 recombinants = (A3) 20X showed { 33 Lac+ / 130 Lac-
1 X showed $8 V_1^R Lac^+$, $8 V_1^R Lac^-$

(A2) $S^R Lac^+$ (2X) numbered ca 21 purplate.

∴ One should have predicted that ½ these would be V_1^R = ca 11.

(A3) Found $S^R V_1^R$ lac^+ = 8 purplate.

1/12/54

Per 2X plate: S^R a Lac$^+$ < V_1^R 11
V_1^S 10

S^R a V_1^R < 8 lac$^+$
8 Lac$^-$.

These experiments suggest that here lac and V_1 are unlinked to each other and segregate independently one of the other. ∴ 3 groups indicated S – Gal (almost always prototypic) Lac; V_1. If parents are

Hfr S^S Gal$^-$ lac$^+$ V_1^R
× ——— ———
F$^-$ S^R Gal$^+$ lac$^-$ V_1^S

Then recombinants are generally S^R Gal$^+$ · $\frac{\text{lac}^+}{\text{Lac}^-}$ · $\frac{V_1^R}{V_1^S}$

but one should test the Gal S character of V_1/lac recombinants for final verification.

The missed recombinants are therefore probably lac$^-$ V_1^R S^R. Do these occur in association with either parent? Would be detected now with the lac$^+$ parent. P1/P2 combinations should be reviewed for other [illegible]

Preliminary mating tests

EMS: #655

A)

W2338 F- line 28A.

(Lac- S^R)

~~W6 F+ line 1.~~

~~W1607~~

W1618

W1655

Mix 10^8 (young cells) each in 10ml broth for 1 hour. Plate out on EMBLac. Test 20 Lac- colonies for F status (x W1607 W-6 ^{180Z} ?).

B) The same in large droplets under oil.

C) Then I will touch single cells together.

Use 1:100 for minute chrps.

L = large chrp.

	A	B	C	D	E	F
1	+L	+L	4bt?	0		
2	0	+L	(1) 116.8 / 27.6	L		
3	+L	+L	(3)	5+(3)+3?		
4	+ chrp L	+L	(2)	1		
5		1 div	L 3+pair	vsm 1+(4)		
6		~	5+pair+chrp	1 vs isolate		
7			3	— 0		
8		L		9 (1 pair) + pair		
9						

D-8 two cells
incipient pairing
1×78
definitely ~~came~~ together
(not nec. mobile)

Single cell method.

DATE: 11/25-26/53. REF: 1083

11/26.

	1	2	3	4	5	6	7	8	9	10
	W2338 x 2341.									

(same pattern?)

whence 7 cells?

	1	2	3	4	5	6	7	8	9
C exp.	3 + frag.	1? + frag	0	1?	## ..	..	..		
found	2- 1+ (3 frag?)	1	1	1	3-2+	4-5+	0		
D exp.	0 + debris	2+ v. large	++	? v.l.	3	..	..		
found	0	4L-1L+	no cool.	1	0	5L-4L+	2L-		

C M [illegible]

	1	2	3	4	5	6	7	8	9
C	4+ ?	1	3	2	3 + pair	2 + pr + clump	3		
found	2- + 1+, 1-?	1-	1-	1-	3- 2+	3-4+ 1pr	0		
D.	0	..	5+3+3	1	1 + 4	1 snake	0	9 + pair	
found	0		1+ 4-	1	0	4+5-	2-	?	sequence?

sequence probably disturbed but argues for moderate eop.

Need to use fewer, better distributed droplets and a marker dye!

No cells in broth side tubes. This can be omitted

Single cell Hfr x F⁻
W 2338 x 2341

1084

DATE: 11/26/53.

REF:

12^{15} — 4:00

5 picked from 10 doublets, direct.

+ = doublet pair attached

	cells seen	EMB lac.	
1	1 + +	2+, 2−	may have grown? or not labeled?
2	1	0	
3	1 pair + doubtful debris	0	
4	7 (clump)	3+, 4−	
5	1 pair.	2+	

all parentals re lac.

(Use eosin, small drops 1:200. Culture too old!)

Cells observed in oil over chamber.

Try ō chamber directly!

ERLively 4/53.

$Lac^+ S^S Hfr \times Lac^- S^R F^-$

Summary of microscopic manipulation experiments with W-1895 × W-1956

I method: Single cells separated from mixture; micro colonies plated on EMB lac.

purpose: To detect recombinants as plates having both Lac^+ & Lac^- cols.

proportion of parental cultures:

expt.	W-1956/W1895	Lac^-/Lac^+	# single cells isolated	plated on:	# plates: cols. Lac^-	Lac^+	missed
5/13/52			6	EMB lac	2	4	0
5/14	(by assay)	3/4	14	"	5	9	0

S character not tested

II method: Small numbers of cells (1–50) were deposited on complete medium agar in holes cut from filter paper. Early growth was observed; then the piece of paper was laid on an EMB lac sm plate.

purpose: To detect recombinants as Lac + or Lac − S^R colonies developing from a known number of cells at a given spot. (& have no record of separating or testing the components of a col.)

expt.	mixture W-1956/W1895	Lac^-/Lac^+	approx. number # fields of cells	total viable cells	plated on	colonies $Lac^- S^R$	Lac^+	$Lac^- S^R$	approx. no. S^S cells (by subtraction)
5/16 & 5/19	omitted (growth failure?)								
5/?/52			7	9	EMB lac sm	3			6
5/21			9	52	"	5			47
5/26			18	140	"	1		1	138
5/28			16	67	"	4			63
5/30			16	79	"	2			77
6/4	1 cc culture/.5 cc		15	80	"	3		1	76
6/7	"		7	45	"	2			43
6/7	"		8	31	EMB lac	1?	8		30
Red Tz added to W-1956									
6/24	1 cc/.5 cc		13	not observed	EMB lac sm	2		2	
6/26	1 cc/.25 cc		13	73	"	3			70
6/30	1/.5		26	95	"	3			92
7/2	5 cc/.5		15	48	"	9		5	34

Summary W-1956 x W-1895

II continued	mainf. by assay		no. fields	no. viable cells	plated on:	Lac⁻ S^R	Lac⁻ S^R	S^S not recovered
exp.	Lac⁻/Lac⁺ (T2 labeled W-1956)							
7/3/52	4.5 cc/.5 cc		14	34	EMB Lac Sm	2	1	31
7/4	4.5/.5 cc	4/5	14	39	"	1		38
7/7	4.5/.5 cc	3/4	8	36	"	0	2	34
7/8	4.5/.5 cc	4/1	8	24	"	1		23
totals			207	852		42	12	802

III method	Single cell isolation of rec marked W-1956 from mixture							
	Lac⁻/Lac⁺		viable cells	plated on:	# plates Lac⁻	Lac⁺	mixed
7/10	4.5 cc/.4 cc	3/1	4	filter paper transf. to EMB Lac Sm	2		2 S^R colo.
7/15	4.5/.4	3/1	8	spread plate EMB Lac	5	1	2
7/17	4.5/.4	7/1	11	EMB Lac	11	0	0
7/22	4.5 red/1 cc + blue T2	3/5	2	"	2	0	0
7/24	4.5 red/1 cc blue T2	1/1	9	"	8		1 * (1 col Lac⁻ S?; +2 col Lac⁺ S^R)
7/29	4.5 red/.4 unmarked	7/1	12	"	12		
Totals			46		40	1	5

#3 only grew. Found them 11/28/53 and tested.

* colonies saved

No trace of 7/15 cultures, more critical

UNIVERSITY OF ILLINOIS
DEPARTMENT OF BACTERIOLOGY
362 NOYES LABORATORY OF CHEMISTRY
URBANA

Nov. 24, 1953

My dear Dr. Lederberg,

Did you really think I would remember? I'm afraid I can't tell any more about the experiments than what is recorded, which isn't much; is it? This is the best I can do by way of summary.

I do remember that in the filter paper transfer experiments, before I started using fz and selecting marked (Lac-) cells, I was plagued by a persistent excess of Lac + and/or S^s cells, those which started to grow under direct observation but failed to produce colonies on sm agar. Several times I assayed the parental mixture to affirm that this excess was greater than might be expected from a higher titer of W-1895. I also tried inoculating fresh broth from the mixture at the time the cells were deposited in the micro-chamber and reassaying at the time the microcolonies were plated, but something always happened to make these assays unreliable, and I don't know what happens to the proportion of the mixture in broth. Do you?

The single cell isolations, 7/15 -7/29/52, seem to have been plated on Lac without sm. I recorded that the Lac + colonies from the mixed plate, 7/24 were tested and found to be S^r. Probably those from the two mixed plates, 7/15, were also tested and found to be S^s. I don't know about the Lac-. It could have been the result you suggest, but I wouldn't base any conclusions on it. I think I saved the cultures, but if you can't find them I don't suppose I could. The Lac + plate in that (7/15) experiment probably arose from an unmarked cell that stuck to the needle and got pulled out by mistake (see drawing of isolation).

Good Luck & amd Happy Thanksgiving to you and Esther and Seymour.

E Helen

Conclusions (11/28/53) apparent morphology of zygotes (normal)

Ethelyn's experiments were directed at a different objective. A few cases of cells giving SR+ recombinants are recorded. Unfortunately most of the experiments involved plating directly to EMB Lac sm. Part of series III was plated on EMB Lac. There were two occasions of Lac+/- from 1-cells. But the two from 7/15 (presumably sisters) were not saved and there is no explicit record of tests for S. ERL thinks they were both Lac^+S^s/Lac^-S^r (bi-parentals). 7/24 were saved, presumably P1 + R1, are being checked now. Her work is therefore not too useful. [My recollection agrees with ERL on the 7/15 expt.]. Do I have any reference in any correspondence?

1-cell exp. cons.

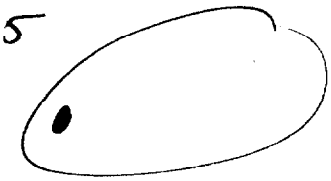
11/17/53.

A. W2344 × W2338. -

1:1:~~5~~5 and dilb 10^{-1}.

B.

5:25 D1 

D5 

E2 

12 others, 2 not formed 1 dirty others o. hold for clones. in chamb

8PM. D1 dried up. D5 dried up E2 dried

∴ n.g. Same with G-H series. no chambers. Legendre weak.

ABC- 4 1-cell drops [ca 15 laid down) But no egg transfer. [egg was rotten egg].

Comments: These experiments i v. flat drops on slides or coverglass. Observation is quite as satisfactory, and keeps the outer surface better (optically). ∴ Dispense i chambers is short term.

These cells are being used in small cell phase, not so good for observation. Onset mitosis in syn is noticed in diplets.

Recommend: ① Use ~~too~~ younger cells ② Use flat depths but add fluid before incubating. ③. Note that in this series oil and slide had been heated and probably desiccated. Water may

11/28/53.

Reinoculate 85B 1:20, 1:100 in Lenessay AM —
Technique: Mark 3x1" slides with india ink. Cover other surface c mineral oil. Add droplets, (culture + cosmis) moderately flat. (After mixing warm add addnl fluid?) Pick up cells or clones by pumping addnl. fluid back and forth in pipette and then expelling this onto agar.

DATE: 11/2 REF: 1086 D.

	1	2	3	4	5	6	7	8	9	10
A.	dirty	dirty	clump 4+?	0 + debris	0	* 1+ dirt ✓	* 1+ dirt ✓ ?all	* 1 v. dense motile	* mass dessicated forms no cells ✓	* 1 + broken? ✓

* add fluid and incubate in jar 2:20 ✓ after.

	1	2	3	4	5	6	7	8	9	10
B.	0	3 v. flat 1 infr. + 1 motile +	1~ ?	0	0	2. + ! sic	0	1 small	0	0

no check.

Inc 2:50. all added small deposits dried out 7:15

	1	2	3	4	5	6	7	8	9	10
B 7:15PM		1 ghost only	n.g.			1 ghost?		ng		

something prob. inhibitory even large amt of drops showed little growth. (errors mated oil?)

Slide was planted in mineral.

	1	2	3	4	5	6	7	8	9	10
A.						2??	ng	0	0	0

over

add droplets with considerable
cells each to test inhibition

Inc. overnight

5 c, 5 s eosin

20-30/drops.

These chicks grew very well (overnight)
but exptl. droplets still empty. Cells invisible?

DATE: 11/29/53. REF:

	1	2	3	4	5	6	7	8	9	10
Remonitate dilutions 1:1000 10AM - 5PM. Pick eosinated droplets, moderate size, immediately.										
A. Pichard. Recovery on Embden	8+ bubble	6	9, dirt	2 ✓ 0	1, †	8 singles	③, ②, 6	4, †	3	1, †
					1−	2−		2+	0	0
B	++	++	++	1, 1 dry?	4	10±	4	3, ③	1	2
				0			1	0	0	0

all parentals

Why such poor recovery? Flat droplets deteriorate?

~~Transfer these notes on single-cell isolation to cytology notebook and renumber.~~

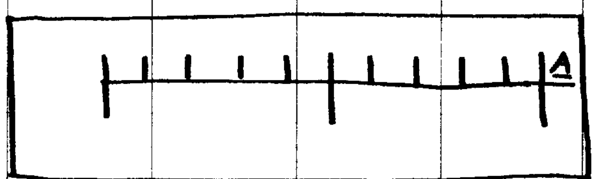
Main problem: Get large young cells. Use ~~Mazola~~ oil droplet as source?

DATE: 11/28/53. REF:

Mix W2344, 2388 .05 + .05 + 10 10 AM.

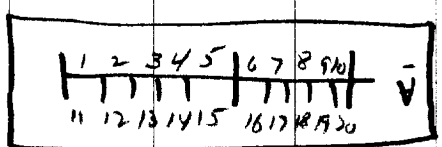
Make large droplets 11:50 AM as source of large bacteria. These droplets noted to have large cells at this time! also remove broth tubes.

Mark 1×3" slides with india ink:



no breuse:

number:



Flame to sterilize. India ink is resistant to oil but not water.

1/1000 dil. at 2:45 ca 10 cells/drop. dil. 1/10 in H_2O; in buffer. No dye really needed.

B Recovered

	1	2	3	4	5	6	7	8	9	10
1-10	5 + 6! ~~X~~	>1 (3) dead	1 [illegible]	1	1 with 4	3	0	2	[illegible]	d.
11-20	✓	0	0	0	2	0	0	0	1	0

use 20 x10 ogtic

add broth to B 4, 5, 14, 19 for clones

Pick B1, 3, 6, 8, 11, 15.

Incubate 4 PM

11. growth limited probably contam

DATE: 12/1/53. REF: SGM.

12/1

Single cells allowed to form vegetative:

B4	—	P1
B5	—	
B14	NG	
B19	+	P2
E2	—	
E9	+	
E13	+	
G5	+	
G10	+	
G14	+	
G20	+	

all pure parentals.

Plates en bloc from 1ml 1/100 assay after 4 hr. further mutation. P1.

B14?, E15, E16, G12 did not develop.

From duit pairs: — = cells seen

B1	0	5 (Resist)	E1	3	4+ = 7	3 !
B3	0	1 small		6	2 − 1+ = 3	3
B6	4+	3		10	1+ 1− = 2	2
B8	4+	2		11	2+ 1− = 3	4
B11	1+	2		14	0 = 0	3
B15	0	2				

all pure tet+

Score = 24/31 Note excess of clones found in some instances! Might be due to subsequent divisions before plating! Repeated washing might be more effective than pumping back and forth!

DATE: REF: 88

	1	2	3	4	5	6	7	8	9	10
D water did'n 4:10	4 (incl +)	X delivered [illegible]				[illegible]	8 [illegible]			
E 1-10 water [illegible] th 10	3 (incl +)	1	++ incl	4+	6	3	10	++	1	2
11-	4	0	1	2+[illegible]	2 [illegible]	3 v [illegible]	0	0	0	X
							dump v.s.			

20 add fluid to E 2, 9, 13. 15, 16, 0 0 Try to [illegible] 1, 6, 10, 11, 14,

Once 4:40

30 capillary too large

	1	2	3
F 505 1	too large dump x	x	++

11

	1	2	3	4	5	6	7	8	9	10
G 1	x	+	6	0	1?	4	5	4	1?	
11	++	1? v. slight +	+	1	0 or v.s.	0	0	0	1	

5:25 add fluid to 5, 10, ~~1~~ died, 14, 20

[illegible]

50

DATE: REF: 1089

	1	2	3	4	5	6	7	8	9	10
L 945	0	+	+	0	0	0	0	+	0	X
	+	0	0	0	0	++	++	0	0	X

4.9. paired!

N. acc. discarded.

① Need better control of numbering directions.

0. Set up sterile drops. overnight.

12/2/53 Plating results: Emb Lac.

H. (direct pick)

1	7+ 5-	1 ++/-	9	1+ 2-		
2	5+ 1-	1 ++/-	10	2+ 2-		
3	4+ 1-		11	3+ 2-		
4	7+		12	5+ 1-		
5	8+ 3-		13	1+ 1-		
6	2+		16	2+	1 ++/- ?	
7	4+		17	4+		
8	2+ 2-		18	2+		

These drops initially were too large for careful observation. Not excluded that rather long cells or pairs were zygotes. However, syngamy may have been after picking: note high yield.

streaks + plates:

I	18	pure lact+	ca 10²		5 : N.G.
	17	"	"	"	
	3	"	"	"	(serially grown cultures show overgrowth of the M-Hfr parent)
	12	"	"	"	
H	14	"	"	"	

88 B. (streaks) 4- 5- 19+

1089

DATE: REF:

	1	2	3	4	5	6	7	8	9	10

4:55 – L 5:05 1 — 1, 0, 0, 0+dirt, 0, 0, 0, 1, 3, 0 ?

– 0, 2, 0, 2+ ?, 0, 1, 0, 0, 2, (0?) (+)

5:10 –

M ~~1, 2+, 1+, 2, 7, 8, 3, 0 dirty, 2, 4, 4~~

Far too dense

N 5:30 1 — 4, 3, (1 / 0), 1+, 1, 1, 0, 1, 1, 1

11 — > debris; 1, 1, +, 0, 0, 0

0, 0, (0) 0?, ~~[illegible]~~ 0 — Inadvertently thrown out

12/1 — 12/2.

0 — 20 sterile drops. 24 hours: 18 sterile

1. had a few clumps of highly pigmented matter

2 had a denser, non coli. thrown out.

Group on ? V1 N ?

DATE: 12/1/53. REF:

		1	2	3	4	5	6	7	8	9	10
Examine drops begin ca 8PM											
H.	3-8:20				14 ca 100 drops v. large.	15 1022 bacteria!? hold room.				19 X	20 O
9P20:	H14	+++			plate ca. 5 cells seen as yeast!						
	H15	–									
	H20	now ++ with a ~~co~~ cocci. May have been noted in first examination! 1-cell ~~conclusions~~ appear justified.									
I.	8:45										
	1	*1	None dv.	ca 1/4 10 clumped	0	*10	0	0	0	0	0
	20	0	0	✓	0	0	0	0	0	0	0
	EMB					~~H5~~					
	11	0	>200	0	0	0	0	✓ 10^2 clumps	✓ $<10^2$ clumps.	0	0.
		0	✓1 ✓	0	0	0	0	1	1	0	
drops are too large for observation.											
	30	Plate 3, 5, 12, 17, 18. Replenish fluid.									
J	9:30	1. ca 100 pathy. contam. 1 group	0	0	0	0	0	>100	>100	0	0
	46	0	++	0	>100 at one edge			drops			

Remember date 24 hours. 12/2: I5: a few "bacteria" but no further growth. others (3, 12, 17, 18 are +++). I8- indefinite shape; others empty by low power.

K.	1	x	++	0	>100	0	++	0	0	0	0
9:35	46	0	++	++	0	0	++ at edge	0	0	0	++

order must have been omitted!! hold overnight

K, order reversed, >24 hours:

	1	2	3	4	5	6	7	8	9	10	
I	+++	0	0	0	+++	0	0	+++	+++	comes	
II.	+++	+++	0	+++	+++	+++	++	0	+++	X	
	(coccoid?)	?	0	1	2	?!?	4	0	1		

6 may have coli +? Plate K5, 8, 10, 14, 16, 19.

Culture #10, 11, 12,

Lac

5 ±

8 ±

10 ng

X 11 ± fewer than others ∴ probably mixed

X 12 ng

16 ± 1 colony

" "

19 ±

why no lac-?

all fits well. why 14 not plated

DATE: REF:

	1	2	3	4	5	6	7	8	9	10
	Fresh cross 10-12N. Remix 1:100/12N – 2:20 = 89-1.									
	Also remix 2:20 1:100, 89-2									
H				5 all rather small						
Tot.	7 incl 1 pt.	3	4							
	deeps too deep									
II					0	0			0	0
	3PM Plate all these at init. but add fluid to and hold. 14, 15, 19, 20.									
I 4PM.	0	0	1	0	0	0	0	0	1+debris	
same as above shallower deep.	0	1	0	0 on ?	0	0	1	1	0	
	add fluid each time. 4:15									
J	1	X	X	X	2	X				
K 4:30	2	0	0	0	1	0	0	1? 2	1	1 ?
	0 deep.	minute + on deep	0	1	2	1? at edge	(4)	0	1	Δ
	add fluid 4:50									
	Try filtered medium?									

12/3/53.

A 1 +2 X 2 0 . 0 0 1 0 0 + 0 + X
11 . 1 0 0 0 0 0
1 dried up
+ 8
X
[illegible] 3.15

B 1 + 0 0 0 [illegible] 0 0 0 0 X 0
1 0 0 0 0 1 X 0 0 0
[illegible] dried up 30 Rev. 5:40

~~C [illegible] ++ 8 5 4 X 6 X~~

D 1

E 1 X 2 ~~0~~ (1) 3 ~~7~~ 3 2 0 0 2 (1)
11 4+ 0 0 ~~0~~2 0 0 +·1 0 0 ~~H~~
add fluid 5:30

Fluid must be added!

Growth by 1 X ✓ ✓ ✓ ✓ ✓ 0 0 ✓ ✓
Py/11 + − + − (+) + − − +
EMBlue 12/5 Plate
2 −, ±
6 ±
9 ±
10 NG
14 all −
16 ±
17
red! ±, −
2, 6, 9, 10, 14, 16, 17
why 2 not plated?

DATE: 12/6/53. REF:

	1	2	3	4	5	6	7	8	9	10

4PM Add cross 2338 x 2344. 1:1000 [illegible] at 12 [illegible] 1:10 [illegible]

D. { 1 0? 8 0 x
11 0

A 1 0 0 0 0 0 1++ 1++ 0 0 800
11 x x 7 >10 0 2++ 0 1 v.sh. x 0

add broth to 6, 7, 10, 16. (4:30) Plate A6, A7, A16

4^{55} [illegible] 2-3 [illegible]

C 21 2++ 1 0 0 1 1 0 0 0 0 0 0 0 1 ++ 1 0 dirt dirt
11 x x x 0 x 0 dirt 2 - 0 0 0

add to 1-10 [illegible]

Plate C1, C8

5:15 1 1 ++ 0 3 0 0 0 1 [illegible] + 3+ 0 [illegible]
B 1 0 0 00 0 0 0 0 0 0 0+dirt 2++ 0 0 x++ 2 : 1 ++ [illegible]
or oil drop?

add fluid to 11-20, 1, 7. 5:30

(P): Plate B1, B17, B20

streak out after growth in broth, EMB lac

11/9/53. A. 6, 7, 16 : all 2344 type = P2
B. 1, 17 P2 only. (B20 P1+P2 No Klobmicro) 2 cells.
C. 1, 8 P2 only D18 P2 only.

(over)

1892 –

DATE: 11/7 REF: 1093

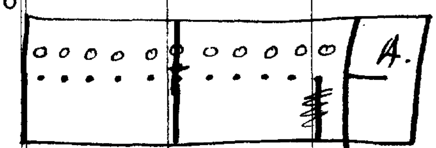
	1	2	3	4	5	6	7	8	9	10
A.	S. fragilis cells overnight. 13, 15 both 1-cell. Latin & bred. both → 10^3									
D B.	1	× dist	~~×~~	0 ?	0	1 ?	1	0	1 ?	0 dist 0
	11	×	0 ?	1	0	0 ?	1	0	1 +d?	0 1?+d?
	10	degenerate; cells small				d (crosses 1:1000 shown)				
E C	1	+	1 +dist	0	0	0	0	?	1	2 ? + +
	11					1+d.	0	×	pair ? 1	+
11/8. E	all but 12, 14 0 by low power.									
	20									
D	only 18 ++ See 1092 results.									
	30	plate A13, 15 for single cell S. fragilis								

#	cells ctd.	types
A 6	1	p2
A 7	1	p2
A 16	2	p2
40		
B 1	1	p2
17	1	p2
20	2	p1 + p2
C 1	2	p2
8	1	p2
D 18	1	p2
50		

DATE: 11/8/53. REF: 1094

W2338 x 2344. ⊗. 8:45 - 10:45. Dilute ca 1:20, incubate large droplet under oil.

2:10 Cells large. Some clumps, chains, etc. Dilute large droplet and proceed per routine. Use pattern: Topside up.



2:35. F 1 · 9:20 — 6 ++ clumps · 9 [illegible] · 1. 0 + d. 30 + d · 1. + large clumps · 8 + 0 (3) ++ · 0 8 2 + · 0 1 + v.s. · dirt 0 + v.s · 3 + large clumped · 8 1 +

whole slide v. dirty — d. X · 00 0 3 + · 0 1 · dirty + · 0 ? + · 0 1 + v.s. · 0 1 ? v. dirty · 0 0 ? 2. + · 3 + ++ · ++ clumps

2:50 add fluid.

3:30 A — X · 0 1. · ++ · ++ · ++ · ++ · 3 · 0 · 1 pale short · 1 v. dirty X

0 · 0 · 5 (1 snake) · 3 · ++ · 2 · Since snake · 6 · 0

3:40 B. 1 — 9 PM X ++ · 0 0 · ? [illegible] · 0 1 ? · 0 0 · 0 0 · 0 1. · 0 0 · 0 0 · 0 0 0

11 — X · 0 0 + VS · 0 v. sh. fungi · 0 0 · 25 VS · 0 20 v.s. · 1. 20 · 0 dirt · 8 ? · 1 at edge prob. lost

add fluid A, B 3:50

Room Temp

8:40 PM. A 2 empty: (13.) ca 10² [illegible] (14:) 60 15 ++ (16) ca 30, some rather small (17) ca 10² 18 50 (19) ++ 20 0 7 (30) 8: 0 9: 0 10 1?

DATE: 1/4/53 REF: 1094/result

	#	2 Cells observed visually	3	4	5 EMB	6 Late	7	8	9 Discordances	10
A	13	5	Isolate		P1 sic	P2	R1			
	14	3	incl pair							
	16	2	v.s.		✓	✓				
	17	5	incl isolate		✓	✓	few (2 : 1 intact +/-)			
10	19	6	...	1		24	1 intact			
	7	3			✓	✓				
B	12	1	vs ?	0						
	15	1	v s	0						
	16	0		0						
20	17	1		0	—					
F	3	1				✓				
	4	1			✓					
	5	3	(1 pr)		✓	✓	no			
	6	1			✓					
30	7	1		0						
	10	1		0						
	12	3				✓				
	14	?			✓					
	15	?		0	✓A					
40	17	1	dirty			✓				
	18	2			✓					
	20	1	pr daughter.		✓					

50

clones had massed to ca 10^2 before plating

4/9/53.

Old cross. Remove c & s aeration. Remove s aeration.
10-3 3-5+

New technique. Score coverglass, examine, break glass and plate fragments (add considerable fluid)

A ~~G+~~ (mainly dry) × ~~1 P~~ 0. ~~smaller P+~~ 0, dirt P 1. ✓ × × × 0 .
B ~~× +++ ×~~ (or 1 tiny) +++ × .
C ~~×~~ .
D ~~0 8' 0 ×~~ .

notes: ~~A optics v poor~~ ?

plate directly:

A ~~A1 × +++ × +++ +++ +++ +++~~

A 4. A4

B. × (1. —) 0 ? 0 × 0? 0 0 0 0
' ? (1 large) ~~fork~~ 1 — ~~large~~ 0 end. Not too clearly done

plate 2, 12, 13 separately and remainder collect.

11/10 → A1 0 B — 0
EMB... A4, P1, P2, R/P1 B2 1 P2
B12 P2 (1) [...]
B13 P1

Technique is therefore OK
A4: possibilities: long cell divided when plated → segregants or A1 ⟺ A4 confused. ∴ This one is doubtful. (over)

DATE: 12/11/53. REF: 1096

	1	2	3	4	5	6	7	8	9	10
	old cons	.	cG together							
A 235 1	x	3	8	4+(4)	6	1	|			
4	6	4+(2)(2)	3	0	4			?		

plate 2, 3, 4, 5, 6 (c) extra fluid, 11, 12, 13, 15 (c)

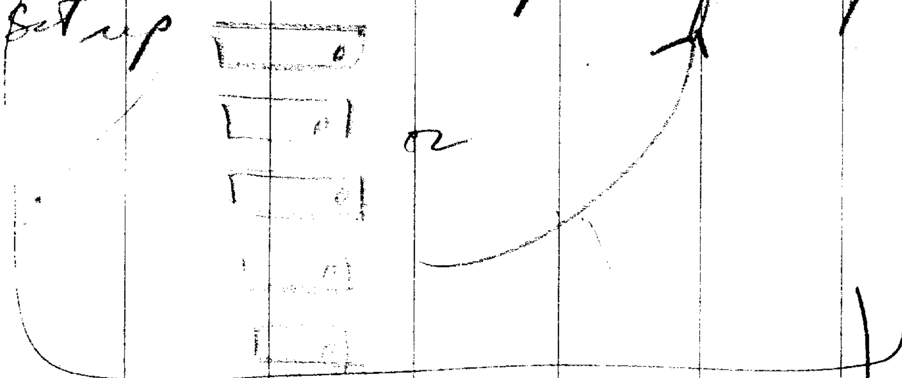
	1	2	3	4	5	6	7	8	9	10
B 3[10]-3[30] 1	~~4~~	~~1+(+)+(2)~~	~~2+(+)~~	~~0~~	~~0~~	2+(+)	•?	•	0	3 sh (+)
11	+	0 ? dup	0	0 +	0	3 sep.	2+ •	0 + ?	0	0, dif

(added fluid).

3[00] (10[30]) 2 mixed dil 1/100 to now

Best to separate squares first.

Set up



C 1 | + | x | x | (2) | | 1 ? 8

keeps mostly n.g. method o.k. Cutting squares takes too long — but them premade! plate c 4 initially

DATE: 12/10/53. REF: 1095–1096

Oil cross, 1230 – 3 PM c/o aviatex 1:100

coverglass method. Stripes of 2-5.

A. 1 3? 1+d. 1.0 + d. 0 of d. 2 — third deg. ✕ 0 ✕ 0 ✕

H 0 0 0 0 2 0 0 0 0 0

Plate 11–15, 16–20, 1, 2, 3, 5

N negative.

1096 10 platings. (If unrecorded, not successfully transferred.) Some difficulties
12/12 in handling the coverglass stripes – at first tried to break previously scored segments, but this was too messy. Here used pre-broken fragments of glass. Plastic, if it could be properly cleaned, would be better as it could be cut in stripes.

	Cells counted →		types:	
A.	2	3	3 P2	— each cell counted for
	4	4+④	0	use extrapolated henceforth.
30	5	6		
	3	1 ghosty?	0	
	6	1 large	0	
	11	6	2 P2	
	12	4+2②	4 P2 + 1 P1	
	13	3	0	
	15	4	2 P2	
40				
B	6	2+1	1 P1	
	7	1 v.s.	0	
	8			
	9			
	10	3 short + 1	2 P2 + 1 P1	
	11	1	1 P2	under oil. —
	13	1	1 P2	—
	14	1	1 P1	all under coverglass. —
50	17	2 + ·	1 P1	
	18	0	0	
	19	1	1 P1	
C	4	②	2 P1	

12/12

Old cross. (24h+). Remove 1:20, ~~9:45~~ 10:30; 1PM, 3:15 PM.

3:35 A. (1+?) (11) m 1-1 (0) (3) (1 eroded) 0 very small × 0 v. flat +

0 0 0 0 0

Plate, add fl., 3:50

P13: E'1 [illegible] colonies

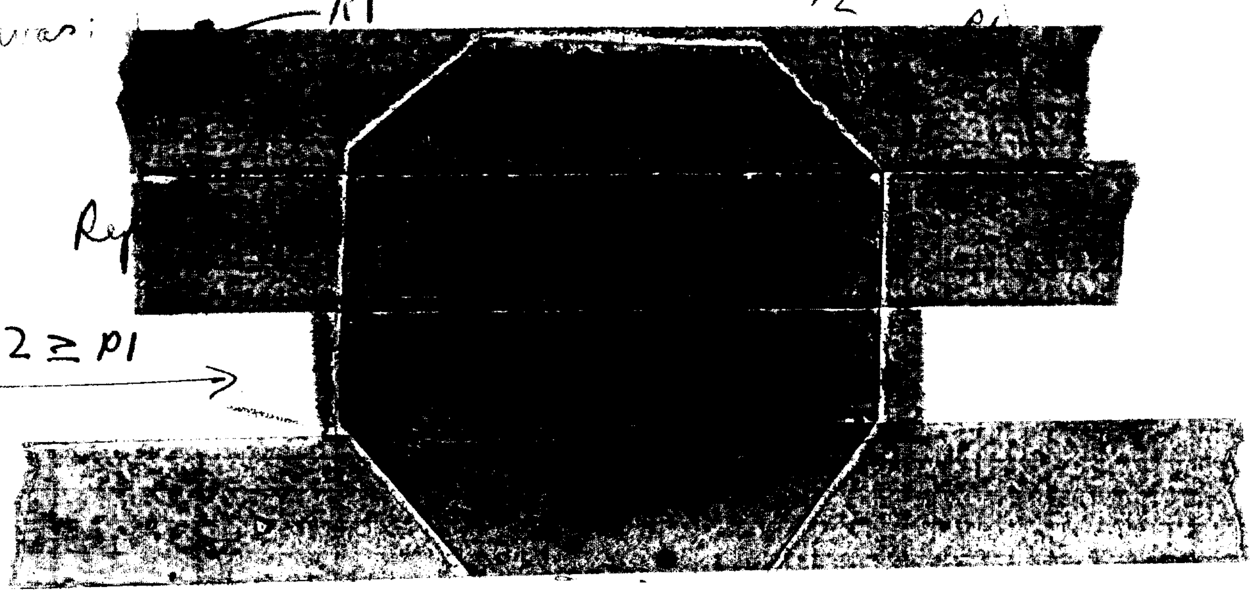
1	1	2P2
2	2(?)	2P1 + P2
3	1 + (2)	3P2 + P1 + (zygote)
4	1	P1
5	3	2P1 + P2
6	1?	0

[illegible] A3, all [illegible] 10% for [illegible]

[illegible]

P2 R1 P2'

X [illegible]



R1 ≐ P2 ≥ P1

12/13
(1 bun)

dil 4PM

A 12+1 1? 0 - + 1? 1+? X 0 0 0

12/14. EMB lac.

	cells	cols.
1	3	1 P1
2	1?	0
3	1	1 P1
4	0	0
5	1?	0
6	1	0

12/14/53.

a). New cross .5 + .5 + 10 from old parent susp. 9:05 AM. — 10 30.
set in H₂O.

20 A. 1 x 0 0 0 0 0 0 ∞ x 1
(lugged) thick

B 1. 3+ pair 0 0 0 x 0 0 0 ∞ P2 ? +
↓ (P1, P2) (?)
(0 v. small. rather large.) ?
possibly not a cell.

C 1 0 8 8 8 3+ 11 2+ 11 0 2 1 0 0
4+. 4 mixed. 5+

2:05

D 0 1 1 + dirt? clump. looser. 0 1. 0 1. 0 1. 1 0 0

2:13 — drops to plating)
12:27

E 5 + 2 + ✓ 1.0 + ? ✓ 0 0 1 v.s.? — 0 . 0 ? ◡ — x 0 —
1:51 —

0 + . 0 8 x 0 ═ 4+ (11 dirt)

5:21

DATE: 12/15. REF: 1099

Platings.

A.

7	1	1 P1
8	+	2 P1

B.

1	4	1P1 + 3P2
9	2 +	2P1
10	1	1P2

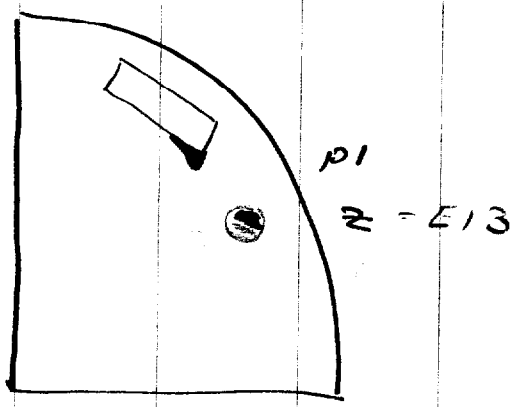
C

1	4+	1P1
2	4	1P1
3	+	1P2
4	5	1P1 + 2P2
5	4	3P2
7	2	1P2
8	1	1P1

why yields so much lower?

D.

32	1	1P2
3	1	0
5	2	1P1
7	1	0
4	3-clump	0
8	1	1P2



E

1	7 + ~~blob~~	3P1 + 3P2
48	1	P2
5	1?	0
8	1?	0
10	1	P2
11	1	P2
13	1	P1 + Z!
17	+	0
16	1	
17	4	4P2

50

large cells recorded. same components.

E13 → P1, R1 No P2 seen. Same mixture and potential toxins.

12/15/53.

12/14. Received cultures from Pomper — lyophil tubes. Incubate in YEx medium.

WY-			
1	62	+++	Pound cells (occ. oval!)
2	63	++	"
3	62-10-19Y	— did not grow out.	(tryptophane, uracil)
4	67-1	++	" (meth, adenine)

12/17. WY-1 grew well and promptly on yeast-sucrose agar
WY 2, 4 grew very poorly initially, but some large colonies suggest incipient adaptation. (probably temper
Hand over to Rubbo for this (There do better at 30 than 37°
After 3 days, WY3 finally grew. Handle as above. ∑ Reversion?)
Transfer from these initial bottles to slants for "cultures as received

WY 6 = dipl S. cerevisiae WY 8, 9 — acriflavine-induced petites (Rubbo).
SOR reports that petites are defective in metabolism of various sugars (cellobiose, rhamnose, maltose, galactose) suggesting adaptive loss generally.

A. Check β-glucosidase in 6 vs 8 grown in glucose, cellobiose.

B. Most sugars, WY 8, 9... showed mighty very poor growth, occasional larger colonies. On EMB Cal, WY8 showed two types of large colonies (fermenters vs slow fermenters). I... and single colonies from EMB Cal of WY8. Also repeat SOR's stations on EMB...

Further tests, nutrition

RL medium base. add niacin 1mg/liter to mix

WY5 (S. fragilis) +++ (~~not~~ previous failure presumably nic requirement)

± metals ... No effect on WY1, WY5 in liquid & moderate medium.

WY1 +++ s/c metals

WY2 +++ confluent flor!

---

48 hours:

WY3 TR+ YNA faint growth. Uracil + TR — TR, YNB only —

WY4 Meth ±

Meth Ad +

Yx +++

Hyd. Cas ++ (enzym.)

HC+pur ++

Meth, pur ±

Meth YNA +++.

hyd.

∴ something in YNA besides adenine. for WY4. another aminoacid?

WY3 unsatisfactory re morphology cavellas growth requirements.

Try adenosine guanosine....

Saccharomyces β-glucosidase

DATE: REF:

1	2	3	4	5	6	7	8	9	10

A. Grow WY6,8 in Nutrient glucose, cellobiose broth.
Growth in cellobiose v. poor for either culture + harvest v. poor.
12/27: test c ONPG. No immediate rx but during several hours, all cultures "adapted" and split off o-n-φOH.

12/29/. Retest. Harvest WY6, 8 from YE agar and resuspend with glucose, cellobiose, 10:50 – 3 in pH 5 buffer + NB.
maltose. ca M/40

Set up 3^25

4^30 faint color only in (8, benzyl, cellobiose!)

v. poor

β-glucosidase indicated

Try S. fragilis / β-galactosidase
5^30 : 6C 6GC 8G — +
8C —
others all —
reaction too feeble to be useful

12/26–29 in Fries sucrose + vit B
Random Pompei mutants. 29°

C) WY 1-2 grow well in F(S), + glucose, + 4 supplements ↓
(WY2 extended, flocculent: large clumps). (Also well at 37°).

WY3-4 failed to grow in F(S) ± glucose ± 4 suppl.
(meth, uracil, trypt, adenine each ca .05 mg/10 ml)

after 2 days delay, WY3 + 4 grow in F(glucose) and more slowly still in F(S). (presumably diploid hybrid) see over

WY4 also grew (poorly) in M, M+Ad but not Ad

Further undoubted ...

D (over)

Burkholder Cross Reaction F(s) agar:

WY	
1	+++
2	+++
3	±
4	±

3 x 4 ± mid scattered prototrophs at intersection.
Yields ca like E coli cross, corresponding body.
Replate these prototrophs as 1100D1

12/30. 3 x 4, both F(s) and F(s)+glucose
fully grown in tubes plate these as 1100 D2, D3.

Comparisons c/s MB, anaerobic supplement in liquid acetate
showed " , cond with oil
maltose greatly better growth than glucose, see W46, W48.
Cf. SDR comparisons. This cannot be ascribed to anaerobic
anaerobic diffusion in maltose case.

1100B

DATE: REF:

1 2 3 4 5 6 7 8 9 10

ca 12/26. Replate WY 6, 8 etc.

12/29: WY6 gives faster growing scalloped colonies
WY8 (1-4 cols): slower growing, smooth. on YE agar

WY6 WY8 WY9.

EMB. Glu all give ++ ferm. reaction

10

WY8, 9 and 8 (1-4) all considerably slower growth.

Gal. all grew more slowly and gave virtually ≡ ferm. as
on plating, 1-4 were indistinguishable as to this Gal!
(Fetal reaction more nearly — than on S.A.'s plate

20

	Ferm	Gr	Ferm	Gr			
Sucrose	±	++	−	+	ø		− +
Maltose	−	+±	−	−			− −
			v. sharp difference in growth!				
Cellobiose		±		−			−

30

Sid's plates did finally show moderate growth on cellobiose ~~+ maltose~~
i.e. papillae possibly better fermenters. Do ~~WY~~ WY 8 on maltose

Conclu: Maltose shows sharpest differential.

40

50